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A comparison of iron limitation of phytoplankton in natural oceanic waters and laboratory media conditioned with EDTA

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Abstract

The solubility of iron in oxic waters is so low that iron can be a limiting nutrient for phytoplankton growth in the open ocean. In order to mimic low iron concentrations in algal cultures, Ethylenediaminetetraacetate (EDTA) is commonly used. The presence of EDTA enables culture experiments to be performed at a low free metal concentration, while the total metal concentrations are high. Using EDTA provides for a more reproducible medium. In this study Fe speciation, as defined by EDTA in culture media, is compared with complexation by natural organic complexes in ocean water where Fe is thought to be limited. To grow oceanic species into iron limitation, a concentration of at least 10^{-4} M EDTA is necessary. Only then does the calculated $[\text{Fe}^{3+}]$ concentrations resemble those found in natural sea water, where the speciation is governed by natural dissolved organic ligands at nanomolar concentrations. Moreover, EDTA influences the redox speciation of iron, and thus frustrates research on the preferred source of Fe-uptake, Fe(III) or Fe(II), by algae. Nowadays, one can measure the extent of natural organic complexation in sea water, as well as the dissolved Fe(II) state, and can use ultra clean techniques in order to prevent contamination. Therefore, it is advisable to work with more natural conditions and not use EDTA to create iron limitation. This is especially important when the biological availability of the different chemical fractions of iron are the subject of research. Typically, many oceanic algae in the smallest size classes can still grow at very low ambient Fe and are not easily cultivated into limitation under ambient sea water conditions. However, the important class of large oceanic algae responsible for the major blooms and the large scale cycling of carbon, silicon and other elements, commonly has a high Fe requirement and can be grown into Fe limitation in ambient seawater. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: iron speciation; iron limitation; phytoplankton; EDTA

1. Introduction

The importance of metal speciation for the life and productivity of phytoplankton has been evident,

ever since the famous publication of Sunda and Guillard (1976). They concluded that growth rate inhibition and Cu content in phytoplankton cells were proportional to the cupric ion activity and not to the total dissolved concentration. From the 1970s onwards, research in metal–phytoplankton interaction was, for a large part, focused on pollution and

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on Cu (Sunda and Guillard, 1976; Brand et al., 1986; Zhou and Wangersky, 1989; Zhou et al., 1989; Robinson and Brown, 1991; Rijstenbil et al., 1994; Gerringa et al., 1995). The interaction between the speciation of dissolved Cu and phytoplankton was tested with (Sunda and Huntsman, 1995a) and without the help of Ethylenediaminetetraacetate (EDTA) (Zhou et al., 1989).

Recent research on metal availability to phytoplankton is especially focused on iron and iron limitation (Anderson and Morel, 1982; de Baar et al., 1995; Hutchins, 1995; Wells et al., 1995). Most of the research has been performed in laboratory cultures using a single species of phytoplankton in either a synthetic medium or filtered natural sea water, where EDTA had been added in order to buffer the metal chemistry (Anderson and Morel, 1982; Brand et al., 1983; Sunda and Huntsman, 1995b, 1997; Sunda et al., 1991; Timmermans et al., 1994; Wilhelm et al., 1996). EDTA has a fairly high affinity for transition metals and was introduced as a trace metal buffering reagent for cultures by Hutner et al. (1950) and Myers et al. (1951). EDTA was used in order to detoxify the medium with respect to high metal concentrations and to obtain a constant and controlled supply of iron. Another major advantage of adding EDTA or other chelators is that it is possible to calculate and manipulate the speciation and thus the ionic metal concentrations (Sunda and Guillard, 1976; Sunda and Huntsman, 1995a,b; Jackson and Morgan, 1978; Anderson and Morel, 1982; Brand, 1991; Brand et al., 1983, 1986; Morel and Morel-Laurens, 1983; Boyd et al., 1996). On the other hand, in the past decade there have been many experiments done at sea where the response of the local plankton community to Fe additions was observed in natural sea water without EDTA, or sometimes low additions of EDTA as well (Martin and Fitzwater, 1988; Martin et al., 1991; de Baar et al., 1990; Buma et al., 1991; Coale, 1991; Price et al., 1991; Boyd et al., 1996; Coale et al., 1996a,b).

Although conceptually there is little difference from toxicological research, e.g., Cu as a pollutant, studies on limitation are more complicated by contamination. The element of interest also matters. The oxidation–reduction chemistry of Fe(III) to Fe(II) plays a major role in its geochemistry and biology. Cu is largely present in the Cu(II) state, i.e., reduced

Cu(I) is not very significant. Moreover, the (inorganic) solubility of Fe(III) is very low. These various problems are the reason why EDTA is added to the cultures. With EDTA, the inadvertent contamination of Fe and other metals is masked, the precipitation of Fe(III) is prevented, the complexation by natural organic ligands can be neglected and the speciation within the artificial medium can be calculated.

Among experimentalists using EDTA media, Anderson and Morel (1982) were aware of the deviations from natural waters. Already in 1982 they were able to initially grow algae into iron limitation without the addition of synthetic chelators. This was followed by a second stage where judiciously chosen types of chelators were applied in an attempt to manipulate Fe speciation in a controlled manner. For such well-defined conditions, imposed by a large excess of various chelators (EDTA, CDTA, DTPA, NTA), in the absence of light, the iron uptake rate was a function of the activity of the Fe^{3+} ion. However, in the absence of chelators, some intriguing trends were found, at quite high overall Fe concentrations ($\sim 10^{-7}$ M) compared to now known values of 10^{-9} M or less in natural oceanic waters. Throughout the years, in many more experiments by various research groups an artificial chelator, mostly EDTA, was always used to fix or influence Fe speciation in culture experiments. However EDTA added at high concentrations changes the composition of sea water considerably. Morel and Morel-Laurens (1983) stated this unambiguously: “It is an often poorly appreciated paradox that to mimic the effects of metals at nanomolar and lower concentrations in oceanic water with very low organic content it is in fact necessary to use culture media containing micromolar concentration of metals and artificial organic ligands.” Moreover, a controlled and constant supply of nutrients might be relevant for model-physiological studies, but for extrapolation to the ever-changing dynamic environment in the sea, it bears little relevance.

Until recently this paradox remained both unavoidable and poorly recognised. For example, the currently prevailing paradigm is that Fe' (that is the sum of dissolved inorganic Fe(III) species) is the primary parameter for Fe uptake by algae (Sunda and Huntsman, 1997). This paradigm only applies to

the EDTA controlled medium from which it was derived, and may not be relevant for natural waters. However, in the past few years it has become possible not only to detect dissolved Fe at realistic concentrations of 10^{-9} M or less (Landing and Bruland, 1981; Gordon et al., 1982; Obata et al., 1993; de Jong et al., 1998; for complete overview see review of de Baar and Boyd, 1999), but also to actually measure its organic complexation and redox speciation (oxidation states) (Gledhill and Van den Berg, 1994, 1995; King et al., 1995; Rue and Bruland, 1995, 1997; Van den Berg, 1995; Wu and Luther, 1995; Aldrich and Van den Berg, 1998). When these new techniques can be applied, EDTA is not required any more to calculate metal speciation, or circumvent inadvertent contamination. Further to this, EDTA, when added in high concentrations, seriously interferes with the new speciation techniques.

In this paper we discuss the speciation of iron controlled by EDTA additions in laboratory cultures and compare this to natural conditions.

2. Chemistry and speciation of Fe

The concentration of iron in sea water is extremely low and its chemical speciation, and thus probably its biological availability, depends on the existence of dissolved organic ligands and the presence of light. Below, we describe what can be expected on theoretical grounds, firstly for a dark inorganic ocean, then for a dark organic ocean and finally, for the illuminated euphotic zone.

In a dark inorganic ocean in equilibrium with the atmosphere, iron is predominantly present as Fe(III). The solubility of Fe(III) depends on the assumed particulate Fe-oxide form in equilibrium with sea water, and the predominant dissolved inorganic iron(III) species being either $\text{Fe}(\text{OH})_3^0$ (Millero et al., 1995) or $\text{Fe}(\text{OH})_2^+$ (Kuma et al., 1996; Millero, 1998). There seems to be a consensus evolving now about the solubility of solid iron(III) species and the conditional stability constants of the dissolved inorganic species (Kuma et al., 1996; Millero, 1998) (Table 1). Colloids (of either inorganic or organic nature) further complicate the sea water chemistry of Fe (Wells et al., 1995). For the current paper focus-

Table 1

The predicted overall equilibrium concentration of Fe(III) in sea water for the three commonly assumed solid phases, and the different assumed dissolved species $^*\text{Fe}(\text{OH})^{2+}$, $\text{Fe}(\text{OH})_2^+$, $\text{Fe}(\text{OH})_3^0$, $\text{Fe}(\text{OH})_4^+$; $^{**}\text{Fe}(\text{OH})^{2+}$, $\text{Fe}(\text{OH})_2^+$ with respect to amorphous $\text{Fe}(\text{OH})_3$; $^{***}\text{Fe}(\text{OH})^{2+}$, $\text{Fe}(\text{OH})_2^+$ and organic ligands with respect to amorphous $\text{Fe}(\text{OH})_3$

	$\text{Fe}(\text{OH})_3(\text{s})$	$\alpha \text{FeOOH}(\text{s})$	$\text{Fe}_2\text{O}_3(\text{s})$
Millero et al., 1995*	12×10^{-9}	3.8×10^{-13}	2.7×10^{-13}
Kuma et al., 1996**	$0.07-0.2 \times 10^{-9}$		
Kuma et al., 1996;	$0.2-0.6 \times 10^{-9}$		
Millero, 1998***			

ing on EDTA effects, these colloids are being ignored for the sake of simplicity and brevity, but may have to be dealt with in future considerations of both EDTA medium (Litter and Blesa, 1992; Nowack and Sigg, 1997) and natural waters.

The following mass balance of dissolved Fe(III):

$$[\text{Fe}_t]_{\text{diss}} = [\text{Fe}^{3+}] + [\text{Fe}(\text{OH})^{2+}] + [\text{Fe}(\text{OH})_2^+] + [\text{Fe}(\text{OH})_3^0] + [\text{Fe}(\text{OH})_4^+] \quad (1)$$

can be rewritten by rewriting the conditional stability constant:

$$K' = \frac{[\text{FeL}_n]^{3-nx}}{[\text{Fe}^{3+}][\text{L}^{x-}]^n} \quad (2)$$

in which L stand for ligand, here (OH^-), into:

$$[\text{FeL}_n]^{3-nx} = K'[\text{Fe}^{3+}][\text{L}^{x-}]^n \quad (3)$$

defining α as:

$$K'[\text{L}^{x-}]^n = \alpha \quad (4)$$

which can be used in the mass balance as follows:

$$[\text{FeL}] = \alpha[\text{Fe}^{3+}]. \quad (5)$$

The mass balance then becomes:

$$\begin{aligned} [\text{Fe}_t]_{\text{diss}} &= [\text{Fe}^{3+}] + \alpha_1[\text{Fe}^{3+}] + \alpha_2[\text{Fe}^{3+}] \\ &\quad + \alpha_3[\text{Fe}^{3+}] + \alpha_4[\text{Fe}^{3+}] \\ &= [\text{Fe}^{3+}] + \alpha_i[\text{Fe}^{3+}] \end{aligned} \quad (6)$$

in which α_i is called the inorganic side reaction coefficient, the sum of the side reaction coefficients of the individual inorganic species, being the value determining the dissolved Fe(III) concentration. This

value varies in the literature about organic complexation of Fe between 10^{10} and $10^{11.9}$ (Rue and Bruland, 1995 used 10^{10} , Rue and Bruland, 1997 10^{11} , Gledhill and Van den Berg, 1994 and Van den Berg, 1995 $10^{11.3}$, Nolting et al., 1998 $10^{11.9}$) and is 10^{10} according to the work of Kuma et al. (1996). For the current study, the choice of inorganic side reaction coefficient is less relevant and we have chosen to follow the suggestion of Kuma et al. (1996) which was also assessed by Millero (1998) by means of model calculations resulting in a solubility of $\text{Fe}(\text{OH})_3(\text{s})$ of 0.2–0.6 nM (Table 1). In the model calculations (Appendix A) only the species $\text{Fe}(\text{OH})^{2+}$ and $\text{Fe}(\text{OH})_2^+$ have been used to calculate α_i (Millero, 1998).

The real ocean also contains organic material. The concentration of Dissolved Organic Carbon (DOC) is now known to be quite uniform around about 40 μM C in deep waters with increases to at most about 80 μM C in plankton blooms in surface waters (Carlson and Ducklow, 1995; Thomas et al., 1995; Pelzer and Hayward, 1996; Wiebinga and de Baar, 1998). The concentrations of Fe complexing ligands are much lower and more variable from about 0.5 to 12 nM Fe equivalents. The conditional stability constants of the complexes of Fe(III) with these ligands or chelators are high and variable over 4 orders of magnitude between 10^{20} and 10^{24} (Gledhill and Van den Berg, 1994; Rue and Bruland, 1995, 1997; Van den Berg, 1995; Nolting et al., 1998). Thus Eq. (6) becomes:

$$\begin{aligned} [\text{Fe}_t]_{\text{diss}} &= [\text{Fe}^{3+}] + [\text{Fe}(\text{OH})^{2+}] + [\text{Fe}(\text{OH})_2^+] \\ &\quad + \alpha[\text{FeL}_o] \\ &= [\text{Fe}^{3+}] + \alpha_i[\text{Fe}^{3+}] + \alpha_o[\text{Fe}^{3+}] \end{aligned} \quad (7)$$

'i' denotes inorganic, 'o' organic.

According to the measured ligand characteristics by Gledhill and Van den Berg (1994), Rue and Bruland (1995; 1997), Van den Berg (1995), and Nolting et al. (1998), the organic side reaction coefficient α_o of Fe(III) varies between $10^{11.7}$ and $10^{14.2}$. This is only about three orders of magnitude variation, apparently the 10-fold variation in concentration of the ligands somewhat compensates the variation of stability constants. When now comparing this organic α_o with the lower inorganic side reaction α_i

of 10^{10} , the equilibrium $[\text{Fe}^{3+}]$ will be lowered. This furthermore increases the solubility of Fe(III) versus solid phases, depending on the ligand concentration up to 1 nM in open ocean waters (Kuma et al., 1996; Millero, 1998) (Table 1).

However, this mass balance (Eq. (7)) still does not represent reality. In the surface layer of the ocean, sunlight affects speciation of iron down to about 100 m depth (Wells et al., 1991b). By energy input of light, Fe(III) is photo-reduced to Fe(II). Radicals, OH, Br_2^- , CO_3^- , R^\cdot , O_2^- and its product H_2O_2 , are also formed of which the last two are thought to be very important in the process of iron oxidation (Moffett and Zika, 1987; Palenik et al., 1991; Faust, 1994; Hoigné et al., 1994; Miller et al., 1995; Voelker and Sedlak, 1995). Colloids, particles and dissolved organic Fe(III) substances are thought to be reduced to Fe(II) (Wells et al., 1991b; Waite and Szymczak, 1993; Waite et al., 1995; Johnson et al., 1994; Miller and Kester, 1994). Thus far, the description relied on the hypothesis of equilibrium, yet by incorporating the redox reactions in the mass balance, we must leave the principle of equilibrium. The mass balance (Eq. (7)) can be extended to:

$$\begin{aligned} [\text{Fe}_t]_{\text{diss}} &= [\text{Fe}^{3+}] + [\text{Fe}(\text{OH})^{2+}] + [\text{Fe}(\text{OH})_2^+] \\ &\quad + \Sigma[\text{FeL}_o] + [\text{Fe}^{2+}] \\ &= [\text{Fe}^{3+}] + \alpha_i[\text{Fe}^{3+}] + \alpha_o[\text{Fe}^{3+}] \\ &\quad + \tau[\text{Fe}^{3+}] \end{aligned} \quad (8)$$

where $[\text{Fe}^{2+}] = \tau[\text{Fe}^{3+}]$.

For the purpose of this paper we adopted a simple description of $\tau \approx (k_o/k_r)I$. τ is a variable determined by irradiance I and its wavelength, and inversely related to the different rate constants of photo-reduction (k_r) of Fe(III) colloids, oxides, dissolved Fe(III)-organic substances or inorganic Fe(III) species and related to the rate constant of re-oxidation to Fe(III) (k_o) which depends also on the concentration of radicals formed (Moffett and Zika, 1987; Faust, 1994; Hoigné et al., 1994).

The concentration of Fe^{2+} heavily depends on the reaction kinetics of the reoxidation. According to Johnson et al. (1994), the Fe(II) concentration is very low (10^{-13} M) because of this fast oxidation. In a controlled laboratory experiment maximum Fe(II)

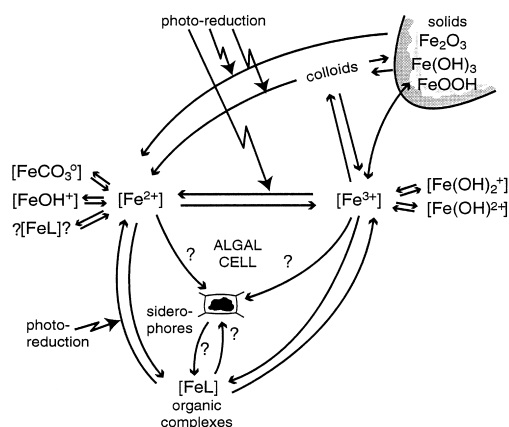


Fig. 1. Schematic representation of the speciation of iron in natural sea water and the possible uptake pathways of iron by an algal cell.

concentrations of 4–8% of total dissolved Fe were measured (Miller et al., 1995). In an estuarine environment with high Fe content during algal blooms 15–20% of total dissolved Fe was Fe(II) due to reduction by organic substances probably excreted by the blooming algae (Kuma et al., 1992). Whereas Zhuang et al. (1995) found that Fe(II) accounts for 50–74% of the total filterable Fe in coastal surface sea water, and Hong and Kester (1986) found that 70% (inshore) to 40% (offshore) of all dissolved Fe was present as Fe(II) near the coast of Peru. Therefore, we can conclude that τ in Eq. (8) can reach values comparable to the sum of the organic side reaction α_o .

The speciation of iron and the possible pathways of iron uptake by an algal cell are schematically represented in Fig. 1.

3. Calculated Fe speciation in sea water with EDTA

The above recent observations and insights of organic complexes and the reduced Fe(II) state of natural sea water now allows assessment of the effect of EDTA addition in previous and ongoing experiments. Based on literature data we investigated the influence of EDTA addition on the chemistry of batch cultures. Data were selected for cultures in

which algae were grown into iron limitation, in artificial and natural sea water media. We selected those studies for which the publications provided enough information in order to calculate iron speciation in the media (EDTA concentrations, total dissolved iron concentrations, pH, salinity). One problem is the lack of information about the possible effects of the pretreatment of the media such as addition of nutrients, and subsequently the risk of contamination, also cleaning with Chelex, and the possible introduction of organic material with active groups for Fe. We ignored the consequences of these treatments.

We assumed that three Fe–EDTA complexes were formed, FeEDTA, Fe(OH)EDTA and Fe(OH)₂EDTA, and used the conditional stability constants given by MINEQL (Secher and McAvoy, 1992) at $S = 35$ (Appendix A), obtaining the following mass balance for iron:

$$\begin{aligned}
 [\text{Fe}]_{\text{diss}} &= [\text{Fe}^{3+}] + [\text{Fe}(\text{OH})^{2+}] + [\text{Fe}(\text{OH})_2^+] \\
 &+ \Sigma[\text{FeL}_o] + [\text{Fe}^{2+}] + [\text{FeEDTA}] \\
 &+ [\text{Fe}(\text{OH})\text{EDTA}] + [\text{Fe}(\text{OH})_2\text{EDTA}] \\
 [\text{Fe}]_{\text{diss}} &= [\text{Fe}^{3+}] + \alpha_i[\text{Fe}^{3+}] + \alpha_o[\text{Fe}^{3+}] \\
 &+ \tau[\text{Fe}^{3+}] + \alpha_{\text{EDTA}}[\text{Fe}^{3+}] \quad (9)
 \end{aligned}$$

in which α_{EDTA} is the sum of the alpha values of the three complexes formed with EDTA.

When natural sea water was used, no information was available on natural occurring dissolved organic ligands. We did not make any assumption about parameters of these ligands since the sea water was treated by Chelex or suffered some other pretreatment and so the composition and/or existence of dissolved organic ligands may have changed considerably. We assumed that the algae in the cultures did not produce any ligands and in case of media made of natural sea water, that EDTA overruled other natural occurring organic ligands. In none of the selected studies was information given about the reduced state of Fe, thus Fe(II) was not taken into account, obtaining the following mass balance for iron:

$$[\text{Fe}]_{\text{diss}} = [\text{Fe}^{3+}] + \alpha_i[\text{Fe}^{3+}] + \alpha_{\text{EDTA}}[\text{Fe}^{3+}] \quad (10)$$

Table 2

(A) Calculated minimum $p[\text{Fe}^{3+}]$ in culture media determined by the added [EDTA] and Fe (Eq. (10) and Appendix A). The minimum added Fe concentrations in the cultures used in the calculations are, respectively: 10, 1, 0.1, 1.2, 5.1, 82 nM. $\alpha_{\text{EDTA}} = \sum K'_i [\text{EDTA}^{4-}]$, i representing three Fe EDTA complexes (see Appendix A). The logarithm of inorganic side reaction coefficient ($\log \alpha_i$) used is 10

Authors	Added [EDTA] (M)	$\log \alpha_{\text{EDTA}}$	Minimum $p[\text{Fe}^{3+}]$
Cultures			
Anderson and Morel, 1982	10^{-5}	12.79	21.2
Brand et al., 1983	10^{-4}	13.79	23.2
Brand, 1991	10^{-7}	10.79	21.2
Sunda and Huntsman, 1995b	10^{-4}	13.79	22.7
Wilhelm et al., 1996	0.8×10^{-4}	13.39	22.4
Muggli and Harrison, 1996a	10^{-6}	11.75	19.2

(B) Calculated minimum $p[\text{Fe}^{3+}]$ in natural sea water calculated with the measured complexation characteristics of natural organic ligand groups (Eq. (7)). $\alpha_o = \sum K_{\text{FeL}} * [L_i]$, i representing one or two dissolved organic ligands. α_o is not corrected when another α_i is used by the authors. The natural organic complexation was measured in the Mediterranean (Van den Berg, 1995), the Central N Pacific (Rue and Bruland, 1995), the Equatorial Pacific (Rue and Bruland, 1997) and in the Antarctic Pacific (Nolting et al., 1998)

	mean $\Sigma[L]$ Meq of Fe	$\log \alpha_o$	Minimum $p[\text{Fe}^{3+}]$
Natural organic complexation			
Van den Berg, 1995	7×10^{-9}	11.5–14.2	22.1
Rue and Bruland, 1995	1.9×10^{-9}	13.7	23.5
Rue and Bruland, 1997	0.5×10^{-9}	14.2	24.9
Nolting et al., 1998	6×10^{-9}	12.6–13.7	22.7

We assumed that these dissolved iron species were in equilibrium, that the alpha factors governed the distribution over the species.

We assumed that α_i of Fe(III) was 10^{10} (Millero, 1998) and used MINEQL ($S = 35$) (Appendix A) to obtain conditional stability constants for the side reaction of EDTA with H, Ca, Mg, Mn, Cu, Zn and Co and for the most important side reactions of the cations (Appendix A). The added EDTA was thus not the concentration available for complexation with iron. When concentrations of the elements above were not known, we used the following concentrations Mn = 1.7 μM , Cu = 40 nM, Zn = 10 nM, Co = 10 nM.

The $[\text{Fe}^{3+}]$ were calculated with Eq. (10) and compared with values occurring in the natural sea water due to measured characteristics of natural dissolved organic ligands using Eq. (7), also neglecting the existence of Fe(II). According to Table 2 concentrations of 10^{-4} M EDTA result in $[\text{Fe}^{3+}]$ concentrations which approach the oceanic and Mediterranean values. This agrees with the reaction of the phytoplankton in the culture experiments. Brand et al.

(1983) and Sunda and Huntsman (1995b) indeed did not observe iron limitation of oceanic species at lower EDTA concentrations. This means that EDTA, due to a large side reaction with other metals (Appendix A), is a weak chelator when compared to the natural dissolved organic complexes in sea water, the latter being present in nanomolar concentrations. Lower concentrations of added EDTA would not have out-competed natural organic ligands, either present in the medium (natural sea water) or excreted by the algae due to their much greater complex stability. Only concentrations of EDTA of 10^{-4} M and higher are capable of doing this and these concentrations are extreme! They are orders of magnitude higher than the concentrations of the natural ligands.

4. Discussion and conclusions

Our assumption, used for the calculation of the $[\text{Fe}^{3+}]$ in Table 2A, that the algae would not produce

any ligands is untrue (Trick et al., 1983; Wilhelm and Trick, 1994; Rue and Bruland, 1997; Butler, 1998), as is the assumption that EDTA would overrule these and natural occurring organic ligands in the sea water medium (Table 2). Thus, speciation in cultures calculated as determined by added EDTA alone is not correct. Only when concentrations of EDTA are 10^{-4} M or higher is the speciation of dissolved Fe controlled by EDTA. With added EDTA concentrations lower than 10^{-4} M in media of natural sea water containing natural organic ligands and ligands produced by the cultured algae $[\text{Fe}^{3+}]$ concentrations may be determined by the natural organic ligand characteristics ($\alpha_0 = \sum K'_i [L_i]$) and could have been influenced by inadvertent Fe contamination. Furthermore, detection of natural ligands by CLE-CSV in the presence of high EDTA is impossible (Gledhill and Van den Berg, 1994; Van den Berg, 1995). This makes it impossible to determine the combined effect of natural organic ligands and EDTA.

Knowledge about the natural speciation of iron is required to provide information about the mechanisms of Fe uptake and what natural occurring species influence this uptake. Anderson and Morel (1982) showed that iron colloids and chelates were photo-reduced to Fe(II) in the presence of light, and that iron uptake was enhanced by photo-reduction (Fig. 1) (Anderson and Morel, 1982; Palenik et al., 1991; Wells et al., 1991b; Kuma et al., 1992; Johnson et al., 1994; Miller and Kester, 1994). According to Kuma et al. (1992), organic substances excreted by the algae were the electron donors for the reduction of iron. We do not know yet the interactions between colloids, Fe(III) complexes and uptake of Fe by phytoplankton. Direct uptake of Fe from colloids is probably not very important (Wells et al., 1991a). However, in coastal areas with high concentrations of colloids and iron binding ligands, the interaction between these species may be important, especially considering photo-reduction processes (Hong and Kester, 1986; Kuma et al., 1992; Zhuang et al., 1995). Since kinetics appeared to play a key role in the uptake process (Jackson and Morgan, 1978; Morel and Morel-Laurens, 1983; Hudson and Morel, 1990, 1993; Palenik et al., 1991; Wells et al., 1991b, 1995; Miller and Kester, 1994; Waite et al., 1995) any disturbance in the reactions will influence the re-

sponse of the phytoplankton. One of these disturbances in the natural ocean causing dis-equilibrium in the Fe chemistry is photo-reduction.

In culture media EDTA is added to impose equilibrium conditions. One of the original classical reasons to use EDTA was preventing precipitation. However, a complicating factor is that EDTA is an efficient bridging-ligand during the Fe(II) catalyzed dissolution of Fe(oxy)hydroxides. Fe complexed with EDTA can also become photodegraded (Anderson and Morel, 1982) releasing Fe^{2+} and formaldehyde (Litter and Blesa, 1992; Sulzberger et al., 1994; Kari et al., 1995). When in culture experiments the influence of light on Fe speciation and Fe uptake by phytoplankton is studied the presence of EDTA will interfere heavily. On one hand, formation of colloids, complexation with natural organic ligands and precipitation of Fe(III) is prevented by EDTA, eliminating possible sources for Fe(III) reduction. On the other hand, the Fe(III)–EDTA complexes can be photodegraded, and form a source of Fe(II).

More recently, some authors applied low concentrations of EDTA to ensure the initial solubility of added iron and assumed that the overall metal speciation would not be changed by its addition. Brand (1991) used EDTA concentrations equal to those of added iron up to 100 nM in his culture experiments. Coale (1991) added 10.2 nM EDTA and extra iron in a ratio Fe:EDTA = 1:1.5 in enrichment experiments in the subarctic Pacific. de Baar et al. (1990), Buma et al. (1991), and Boyd et al. (1996) added iron to in vitro experiments, in a 1:3 and 1:1.5 Fe–EDTA solution in the Weddell and Scotia seas and in the NE subarctic Pacific, respectively. In view of the data in Table 2, the assumption that nanomolar concentrations of EDTA would not change the metal speciation is correct, since EDTA is not a strong chelator in sea water. Muggli and Harrison (1996b) proposed working without EDTA because of a supposed poisonous effect of EDTA at higher concentrations. They therefore conducted another experiment (Muggli and Harrison, 1997) without EDTA and tried to grow two oceanic phytoplankton species into iron limitation and succeeded only for one plankton species.

We know that EDTA has been a very useful tool to influence the dissolved chemical speciation as well as dissolved versus colloidal and particulate

fractions of iron. The use of EDTA resulted in proof of limitation of cell growth by iron (Brand, 1991; Brand et al., 1983; Sunda and Huntsman, 1995b; Wilhelm et al., 1996 and many others) and is an excellent tool to control metal speciation in a reproducible manner when other parameters are being varied (Sunda and Huntsman, 1995b, 1997). This has led to many highly reproducible data sets of plankton growth in laboratory media. However, since it is obvious that knowledge of speciation is essential, the use of EDTA to mimic natural waters is questionable. Extremely high concentrations of EDTA, 10^{-4} M, are necessary to reach natural $[\text{Fe}^{3+}]$. Not only is the organic carbon content raised to unnaturally high values but also the speciation of iron is changed. Nowadays, methods exist to measure the complexation characteristics of the natural organic ligands, as well as redox speciation of iron (Gledhill and Van den Berg, 1994, 1995; King et al., 1995; Rue and Bruland, 1995; Van den Berg, 1995; Wu and Luther, 1995; Aldrich and Van den Berg, 1998), and thus the use of EDTA in order to define metal speciation is not obligatory anymore. As a logical consequence it was decided to perform experiments without EDTA in the project Marine Ecosystems Regulation: Trace Metal and Carbon Dioxide Limitation (MERLIM). This enabled measurements of Fe speciation as well as physiological/molecular response studies. The first results are most promising. In the course of the experiment, increases in ligand concentration were positively related to phytoplankton production (unpublished results).

However, to grow oceanic species in iron limitation without addition of EDTA will require extremely clean conditions and the use of natural sea water without any treatment with Chelex or otherwise.

Avoiding the use of EDTA does not necessarily interfere with relevant iron–phytoplankton research. For years, the most interesting phytoplankton species to use for research on HNLC conditions were deemed oceanic species. It is clear now that many of those species, the small phytoplankton, seem to grow quite near or sometimes at maximum growth rate, despite very low ambient Fe concentrations. Their numbers are being controlled instead by grazers (HNLC conditions = “grazer controlled phytoplankton populations in an iron-limited ecosystem”, cf. Price et al.,

1994), viruses (Suttle and Chan, 1994) or even by some iron stress. This and laboratory studies, without but mostly with EDTA additions (Brand, 1991; Sunda et al., 1991), has led to somewhat of another paradigm that oceanic eukaryotes are adapted to very low Fe abundance and cannot easily be grown into Fe limitation.

On the other hand, as observed in the natural oceanic situation (de Baar et al., 1995) and in virtually all oceanic iron enrichment experiments both in situ (Coale et al., 1996b) and in bottles, the rare, large phytoplankton species such as large chain-forming diatoms show the strongest response upon addition of iron (de Baar and Boyd, 1999). In many ways, the trace metal requirements of these large diatoms resemble those of coastal species, i.e., they have high requirements of the trace nutrient Fe. However, most are truly oceanic or cosmopolitan and in fact responsible for the major oceanic plankton blooms and related export of organic matter and opal into deep waters (Scharek et al., 1999; Smetacek, 1999). So it seems that the latter large oceanic species are highly relevant for iron–phytoplankton research and they can be grown into Fe-limitation relatively easily without using EDTA. Another alternative is the addition of a natural siderophore like deferrioxamine B (Hutchins et al., 1999; Wells, 1999) to induce Fe limitation. The advantage of these siderophores over EDTA is the higher K' for Fe(III), comparable to natural organic ligands. Therefore, the concentration that needs to be added is in the nanomolar range. Moreover, organic speciation, including the siderophore itself, can be measured (M. Boye, personal communication).

Our calculations are based on recent breakthroughs and state of the art literature of the existence and Fe-affinities of natural organic ligands in natural seawater (Gledhill and Van den Berg, 1994; Rue and Bruland, 1995, 1997; Van den Berg, 1995; Nolting et al., 1998). Here one realizes these advanced techniques usually identify one or sometimes at most two ligands, where the inherent detection window of these techniques is a function of chosen competitive ligand addition (e.g., *N*-nitrosonaphthol) and other experimental conditions may play a role. Conceivably, other ligand classes may also exist in natural seawater awaiting assessment and quantification with further evolution of methodology, also for

estimation of molecular structure and weight. With regards to the inorganic speciation and side reaction coefficient, new insight may also evolve leading to some adjustments of the values here assumed in our model calculations. Better insights in the interaction of plankton organisms with chemical speciation, including biotic feedbacks on solution chemistry may also develop and in due course require refining our calculations. Yet clearly, all such further insights and information can only be gained by studying natural seawater.

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Appendix A. Parameters used for the calculations of $[\text{Fe}^{3+}]$

MINEQL + (Secher and McAvoy, 1992) was used for the conditional stability constants at $S = 35$, with the exception of those of the dissolved Fe(III) hydroxides and the calculation of dissociation of carbonic acid. $\text{Fe}(\text{OH})_2^+$ and $\text{Fe}(\text{OH})_2^{2+}$ were used for the inorganic side reaction of Fe (Millero, 1998; Kuma et al., 1996). $\alpha_i = 10^{10}$. Carbonate has been calculated from the total alkalinity (Alk total) and borate concentration according to Roy et al. (1993a, b). For Ca and Mg, only the inorganic side reaction itself ($\alpha_i = 0.5$) is given (obtained from MINEQL +). In this example, total dissolved $[\text{Fe}] = 82 \text{ nM}$ was used, $[\text{EDTA}] = 1 \text{ } \mu\text{M}$. The approximations are used in order to obtain a good estimate of the side reaction of Fe with EDTA, in which the Fe^{3+} concentration is used determined by the added EDTA.

Species	log K'	Species	log K'	CO_3^{2-} calculation		Components	Concentration (M)
$\text{Fe}(\text{OH})_2^{2+}$	-2.6	CoSO_4	1.32	Alk total borate K_{dis} borate	1.88E - 03 3.20E - 06 9.33E - 10	[Ca] [Co] [Cu] [Mg] [Mn] [Zn] [Cl] [SO ₄] [H] [EDTA] [Fe]	1.00E - 02 1.00E - 08 4.00E - 08 5.50E - 02 1.70E - 06 1.00E - 08 0.56 2.90E - 02 1.00E - 08 1.00E - 06 8.20E - 08
$\text{Fe}(\text{OH})_2^+$	-6.00	CoCl^+	-0.09				
		$\text{Co}(\text{OH})_2$	-18.6				
		$\text{Cu}(\text{OH})_2$	-14				
$\text{Fe}(\text{OH})\text{EDTA}^{2-}$	18	CuCO_3	5.55	K^1 ^a	K^2 ^a		
$\text{Fe}(\text{OH})_2\text{EDTA}^{3-}$	8.04	$\text{Cu}(\text{CO}_3)_2^{2-}$	8.65				
FeEDTA^{1-}	24.3	MnCl_2	-0.84	1.12E - 06	7.97E - 10		
HEDTA^{3-}	8.78	MnCl^+	0.02				
$\text{H}_2\text{EDTA}^{2-}$	14.2	MnSO_4	1.03				
H_3EDTA^-	16.2	$\text{Zn}(\text{OH})_2$	-17.2				
H_4EDTA	18	$\text{Zn}(\text{CO}_3)_2^{2-}$	8.45	$[\text{CO}_3^{2-}]$	132E - 06		
CaEDTA^{2-}	10.1	$\text{Zn}(\text{OH})\text{Cl}$	-8.07				
CoEDTA^{2-}	15.7	ZnSO_4	1.15				
CuEDTA^{2-}	16.4	ZnCO_3	4.12				
MgEDTA^{2-}	8.14						
MnEDTA^{2-}	13.4						
ZnEDTA^{2-}	14.1						
(total concentration)/ α_i				Inorganic reaction side coefficient α_i			
InorgCo	9.4E - 09		Ca	0.5			
InorgCu	2.58E - 10		Mg	0.5			
InorgMn	1.8E - 06						
InorgZn	1.31E - 09						

α_i	1.00E + 10	= inorganic side reaction coefficient Fe(III)
α EDTA (pH)	7.04E + 00	= side reaction coefficient EDTA with H^+
α EDTA (other metals)	6.67E + 07	= side reaction coefficient EDTA with other metals
[EDTA ⁴⁻]	1.38E - 14	= third approximation EDTA ⁴⁻
α Fe(EDTA)	1.42E + 12	= third approximation side reaction coefficient Fe with EDTA
[Fe ³⁺]	5.74E - 20	= third approximation ionic iron Fe ³⁺
[Fe _{inorganic}]	5.74E - 10	= inorganic Fe(III) or Fe'
[Fe _{EDTA species}]	8.14E - 08	= sum of Fe EDTA species

^aRoy et al., 1993a,b.

References

- Aldrich, A.P., Van den Berg, C.M.G., 1998. Determination of iron and its redox speciation in seawater using cathodic stripping voltammetry. *Electroanalysis* 10 (6), 369–373.
- Anderson, A.A., Morel, F.M.M., 1982. The influence of aqueous iron chemistry on the uptake of iron by the coastal diatom *Thalassiosira weissflogii*. *Limnol. Oceanogr.* 27, 789–813.
- Boyd, P.W., Muggli, D.L., Varela, D.E., Goldblatt, R.H., Chretien, R., Orians, K.J., Harrison, P.J., 1996. In vitro iron enrichment experiments in the NE Pacific. *Mar. Ecol. Prog. Ser.* 136, 179–193.
- Brand, L.E., 1991. Minimum iron requirements of marine phytoplankton and the implications for the biogeochemical control of new production. *Limnol. Oceanogr.* 36, 1756–1771.
- Brand, L.E., Sunda, W.G., Guillard, R.R.L., 1983. Limitation of marine phytoplankton reproductive rates by zinc, manganese and iron. *Limnol. Oceanogr.* 28, 1182–1198.
- Brand, L.E., Sunda, W.G., Guillard, R.R.L., 1986. Reduction of marine phytoplankton reproduction rates by copper and cadmium. *J. Exp. Mar. Biol. Ecol.* 96, 225–250.
- Buma, A.G.J., de Baar, H.J.W., Nolting, R.F., van Bennekom, A.J., 1991. Metal enrichment experiments in the Weddell–Scotia Seas: effects of iron and manganese on various plankton communities. *Limnol. Oceanogr.* 36, 1865–1879.
- Butler, A., 1998. Acquisition and utilization of transition metal ions by marine organisms. *Science* 281, 207–210.
- Carlson, C.A., Ducklow, H.W., 1995. Dissolved organic carbon in the upper ocean of the central equatorial Pacific Ocean, 1992: daily and fine scale variations. *Deep-Sea Res.* 42, 639–656.
- Coale, K.H., 1991. Effects of iron, manganese, copper and zinc enrichments on productivity and biomass in the subarctic Pacific. *Limnol. Oceanogr.* 36, 1851–1864.
- Coale, K.H., Fitzwater, S.E., Gordon, R.M., Johnson, K.S., Barber, R.T., 1996a. Control of community growth and export production by upwelled iron in the equatorial Pacific Ocean. *Nature* 379, 621–624.
- Coale, K.H., Johnson, K.S., Fitzwater, S.E., Gordon, R.M., Tanner, S., Chavez, F.P., Feriolo, L., Sakamoto, C., Rogers, P., Millero, F., Steinberg, P., Nightingale, P., Cooper, D., Cochlan, W.P., Laundry, M.R., Constantinou, J., Rollwagen, G., Trassvina, A., Kudela, R., 1996b. A massive phytoplankton bloom induced by an ecosystem-scale iron fertilization experiment in the equatorial Pacific Ocean. *Nature* 383, 495–501.
- de Baar, H.J.W., Boyd, P.M., 1999. The role of iron in plankton ecology and carbon dioxide transfer of the global oceans. In: Hanson, R.B., Ducklow, H.W., Field, J.G. (Eds.), *The Dynamic Ocean Carbon Cycle: A Midterm Synthesis of the Joint Global Ocean Flux Study*, Chap. 4. International Geosphere Biosphere Programme Book Series, Cambridge Univ. Press, pp. 61–140.
- de Baar, H.J.W., Buma, A.G.J., Nolting, R.F., Cadée, G.C., Jaques, G., Tréguer, G.J., 1990. On iron limitation of the Southern Ocean: experimental observations in the Weddell and Scotia Seas. *Mar. Ecol. Prog. Ser.* 65, 105–122.
- de Baar, H.J.W., de Jong, J.T.M., Bakker, D.C.E., Löscher, B.M., Veth, C., Bathmann, U., Smetacek, V., 1995. Importance of iron for phytoplankton spring blooms and CO₂ drawdown in the Southern Ocean. *Nature* 373, 412–415.
- de Jong, J.T.M., den Das, J., Bathmann, U., Stoll, M.H.C., Kattner, G., Nolting, R.F., de Baar, H.J.W., 1998. Dissolved iron at subnanomolar levels in the Southern Ocean as determined by ship-board analysis. *Anal. Chim. Acta* 377, 113–124.
- Faust, B.C., 1994. A review of photochemical redox reactions of iron (III) species in atmospheric, oceanic and surface waters: influences on geochemical cycles and oxidant formation. In: Helz, G.R., Zepp, R.G., Crosby, D.G. (Eds.), *Aquatic and Surface Photochemistry*. Lewis Publishers, CRC Press, pp. 3–37.
- Gerringa, L.J.A., Rijstenbil, J.W., Poortvliet, T.C.W., van Drie, J., Schot, M.C., 1995. Speciation of copper and responses of the marine diatom *Ditylum brightwellii* upon increasing copper concentrations. *Aquat. Toxicol.* 31, 77–91.
- Gledhill, M., Van den Berg, C.M.G., 1994. Determination of complexation of iron (III) with natural organic complexing ligands in sea water using cathodic stripping voltammetry. *Mar. Chem.* 47, 41–54.
- Gledhill, M., Van den Berg, C.M.G., 1995. Measurement of the redox speciation of iron in seawater by catalytic cathodic stripping voltammetry. *Mar. Chem.* 50, 51–61.
- Gordon, R.M., Martin, J.H., Knauer, G.A., 1982. Iron in north-east Pacific waters. *Nature* 299, 142–143.
- Hoigné, J., Zuo, Y., Nowell, L., 1994. Photochemical reactions in atmospheric waters: role of dissolved iron species. In: Helz, G.R., Zepp, R.G., Crosby, D.G. (Eds.), *Aquatic and Surface Photochemistry*. Lewis Publishers, CRC Press, pp. 75–84.
- Hong, H., Kester, D.R., 1986. Redox state of iron in the offshore waters of Peru. *Limnol. Oceanogr.* 31, 512–524.
- Hudson, J.M., Morel, F.M.M., 1990. Iron transport in marine phytoplankton: kinetics of cellular and medium coordination reactions. *Limnol. Oceanogr.* 35, 1002–1020.
- Hudson, R.J.M., Morel, F.M.M., 1993. Trace metal transport by marine microorganisms: implications of metal coordination kinetics. *Deep Sea Res.* 40 (1), 129–150.

- Hutchins, D.A., 1995. Iron in the marine phytoplankton community. *Prog. Phycol. Res.* 11, 1–49.
- Hutchins, D.A., Franck, V.M., Brzezinski, M.A., Bruland, K.W., 1999. Inducing phytoplankton iron limitation in iron-replete coastal waters with a strong chelating ligand. *Limnol. Oceanogr.* 44, 1009–1018.
- Hutner, S.H., Provasoli, L., Schatz, A., Haskins, C.P., 1950. Some approaches to the study of metals in the metabolism of microorganisms. *Proc. Am. Philos. Soc.* 94, 152–170.
- Jackson, G.A., Morgan, J.J., 1978. Trace metal–chelator interactions and phytoplankton growth in seawater media: theoretical analysis and comparison with reported observations. *Limnol. Oceanogr.* 23, 268–282.
- Johnson, K.S., Coale, K.H., Elrod, V.A., Tindale, N.W., 1994. Iron photochemistry in sea water from the equatorial Pacific. *Ocean. Mar. Chem.* 46, 319–334.
- Kari, F.G., Hilger, S., Canonica, S., 1995. Determination of the reaction quantum yield for the photochemical degradation for Fe(III)–EDTA: implications for the environmental fate of EDTA in surface waters. *Environ. Sci. Technol.* 29, 1008–1017.
- King, D.W., Lounsbury, H.A., Millero, F.J., 1995. Rates and mechanism of Fe(II) oxidation at nanomolar total iron concentrations. *Environ. Sci. Technol.* 29, 818–824.
- Kuma, K., Nakabayashi, S., Suzuki, Y., Kudo, I., Matsunaga, K., 1992. Photo-reduction of Fe(III) by dissolved organic substances of Fe(II) in seawater during spring blooms. *Mar. Chem.* 37, 15–27.
- Kuma, K., Nishioka, J., Matsunaga, K., 1996. Controls on iron (III) hydroxide solubility in seawater: the influence of pH and natural organic chelators. *Limnol. Oceanogr.* 41, 396–407.
- Landing, W.M., Bruland, K.W., 1981. The vertical distribution of iron in the northeast Pacific. *Eos, Transactions American Geophysical Union* 62, 906, Abstract.
- Litter, M.I., Blesa, M.A., 1992. Photodissolution of iron oxides: IV. A comparative study on the photodissolution of hematite, magnetite, and maghemite in EDTA media. *Can. J. Chem.* 70, 2502–2510.
- Martin, J.H., Fitzwater, S.E., 1988. Iron deficiency limits phytoplankton growth in the north-east Pacific subarctic. *Nature* 331, 341–343.
- Martin, J.H., Gordon, R.M., Fitzwater, S.E., 1991. The case for iron. *Limnol. Oceanogr.* 36, 1793–1802.
- Miller, W.L., Kester, D., 1994. Photochemical iron reduction and iron availability in seawater. *J. Mar. Res.* 52, 325–343.
- Miller, W.L., King, D.W., Lin, J., Kester, D.R., 1995. Photochemical redox cycling of iron in coastal seawater. *Mar. Chem.* 50, 63–77.
- Millero, F.J., 1998. Solubility of Fe(III) in seawater. *Earth Planet. Sci. Lett.* 154, 323–329.
- Millero, F.J., Yao, W., Aicher, J., 1995. The speciation of Fe (II) and Fe (III) in natural waters. *Mar. Chem.* 50, 21–39.
- Moffett, J.W., Zika, R.G., 1987. Reaction kinetics of hydrogen peroxide with copper and iron in seawater. *Environ. Sci. Technol.* 21, 804–810.
- Morel, F.M.M., Morel-Laurens, N.M.L., 1983. Trace metals in phytoplankton in the oceans: facts and speculations. In: Wong, C.S., Boyle, E., Bruland, K.W., Burton, J.D., Goldberg, E.D. (Eds.), *Trace Metals in Sea Water*. Plenum, New York.
- Muggli, D.L., Harrison, P.J., 1996a. Effects of nitrogen source on the physiology and metal nutrition of *Emiliania huxleyi* grown under different iron and light conditions. *Mar. Ecol. Prog. Ser.* 130, 255–267.
- Muggli, D.L., Harrison, P.J., 1996b. EDTA suppresses the growth of oceanic phytoplankton from the Northeast Subarctic Pacific. *J. Exp. Mar. Biol. Ecol.* 205, 221–227.
- Muggli, D.L., Harrison, P.J., 1997. Effects of iron on two oceanic phytoplankters grown in natural NE subarctic Pacific seawater with no artificial chelators present. *J. Exp. Mar. Biol. Ecol.* 212, 225–237.
- Myers, J., Philips, J.N. Jr., Graham, J.-R., 1951. On the mass culture of algae. *Plant Physiol.* 26, 539–548.
- Nolting, R.F., A Gerringa, L.J., Swagerman, M.J.W., Timmermans, K.R., de Baar, H.J.W., 1998. Fe III speciation in the high nutrient, low chlorophyll Pacific region of the Southern Ocean. *Mar. Chem.* 62, 335–352.
- Nowack, B., Sigg, L., 1997. Dissolution of Fe(III) (hydr)oxides by metal–EDTA complexes. *Geochim. Cosmochim. Acta* 61, 951–963.
- Obata, H., Karatani, H., Nakayama, E., 1993. Automated determination of iron in seawater by chelating resin concentration and chemiluminescence detection. *Anal. Chem.* 65, 1524–1528.
- Palenik, B., Price, N.M., Morel, F.M.M., 1991. Potential effects of UV-B on the chemical environment of marine organisms: a review. *Environ. Pollut.* 70, 117–130.
- Pelzer, E.T., Hayward, N.A., 1996. Spatial and temporal variability of total carbon along the 140°W in the equatorial Pacific Ocean in 1992. *Deep-Sea Res., Part II* 43, 1155–1180.
- Price, N.M., Anderson, L.F., Morel, F.M.M., 1991. Iron and nitrogen of equatorial Pacific plankton. *Deep-Sea Res.* 38, 1361–1378.
- Price, N.M., Ahner, B.A., Morel, F.M.M., 1994. The equatorial Pacific Ocean: grazer-controlled phytoplankton populations in an iron-limited ecosystem. *Limnol. Oceanogr.* 39, 520–534.
- Rijstenbil, J.W., Derksen, J.W.M., Gerringa, L.J.A., Poortvliet, T.C.W., Sandee, A., van den Berg, M., van Drie, J., Wijnholds, J.A., 1994. Oxidative stress induced by copper: defense and damage in the marine planktonic diatom *Ditylum brightwellii* (West) Grunow, grown in continuous cultures with high and low zinc levels. *Mar. Biol.* 119, 583–590.
- Robinson, M.G., Brown, L.N., 1991. Copper complexation during a bloom of *Gymnodinium sanguineum* Hirasaka (Dinophyceae) measured by ASV. *Mar. Chem.* 33, 105–118.
- Roy, R.N., Roy, L.N., Lawson, M., Vogel, K.M., Porter-Moore, C., Davis, W., Millero, F.J., 1993a. Thermodynamics of the dissociation of boric acid in seawater at $S = 35$ from 0 to 55°. *Mar. Chem.* 44, 243–248.
- Roy, R.N., Roy, L.N., Vogel, K.M., Porter-Moore, C., Pearson, T., Good, C.E., Millero, F.J., Campbell, D.M., 1993b. The dissociation constants of carbonic acid in seawater at salinities 5 to 45 and temperatures 0 to 45°C. *Mar. Chem.* 44, 249–267.
- Rue, E.L., Bruland, K.W., 1995. Complexation of iron (III) by natural organic ligands in the Central North Pacific as determined by a new competitive ligand equilibration/adsorptive

- cathodic stripping voltammetric method. *Mar. Chem.* 50, 117–138.
- Rue, E.L., Bruland, K.W., 1997. The role of organic complexation on ambient iron chemistry in the equatorial Pacific Ocean and the response of a mesoscale iron addition experiment. *Limnol. Oceanogr.* 42, 901–910.
- Scharek, R., Tupas, L.M., Karl, D.M., 1999. Diatom fluxes to the deep sea in the oligotrophic North Pacific gyre at Station ALOHA. *Mar. Ecol. Prog. Ser.* 182, 55–67.
- Secher, W.D., McAvoy, D.C., 1992. MINEQL+: a software environment for chemical equilibrium modeling. *Computers, Environment and Urban Systems* 16, 65–76.
- Smetacek, V., 1999. Diatoms and the ocean carbon cycle. *Protist* 150, 25–32.
- Sulzberger, B., Laubscher, H., Karametaxas, G., 1994. Photoredox reactions at the surface of iron(III) (hydr)oxides. In: Helz, G.R., Zepp, R.G., Crosby, D.G. (Eds.), *Aquatic and Surface Photochemistry*. Lewis Publishers, CRC Press, pp. 53–73.
- Sunda, W., Guillard, R.R.L., 1976. The relationship between cupric ion activity and the toxicity of copper to phytoplankton. *J. Mar. Res.* 134, 511–529.
- Sunda, W.G., Huntsman, S.A., 1995a. Regulation of copper concentration in the oceanic nutricline by phytoplankton uptake and regeneration cycles. *Limnol. Oceanogr.* 40, 132–137.
- Sunda, W.G., Huntsman, S.A., 1995b. Iron uptake and growth limitation in oceanic and coastal phytoplankton. *Mar. Chem.* 50, 189–206.
- Sunda, W.G., Huntsman, S.A., 1997. Interrelated influence of iron, light and cell size on marine phytoplankton growth. *Nature* 390, 389–392.
- Sunda, W.G., Swift, D.G., Huntsman, S.A., 1991. Low iron requirement for growth in oceanic phytoplankton. *Nature* 351, 55–57.
- Suttle, C.A., Chan, A.M., 1994. Dynamics and distribution of Cyanophages and the effect on marine *Synechococcus* spp. *Appl. Environ. Microbiol.* 60, 3167–3174.
- Thomas, C., Cauwet, G., Minster, J.F., 1995. Dissolved organic carbon in the equatorial Atlantic Ocean. *Mar. Chem.* 49, 155–169.
- Timmermans, K.R., Stolte, W., de Baar, H.J.W., 1994. Iron mediated effects on nitrate reductase in marine phytoplankton. *Mar. Biol.* 121, 389–396.
- Trick, C.G., Andersen, R.J., Price, N.M., Gillam, A., Harrison, P.J., 1983. Examination of hydroxamate-siderophore production by neritic eukaryotic marine phytoplankton. *Mar. Biol.* 75, 9–17.
- Van den Berg, C.M.G., 1995. Evidence for organic complexation in seawater. *Mar. Chem.* 50, 139–159.
- Voelker, B.M., Sedlak, D.L., 1995. Iron reduction by photoreduced superoxide in seawater. *Mar. Chem.* 50, 93–110.
- Waite, T.D., Szymczak, R., 1993. Particulate iron dynamics in surface waters of the Eastern Caribbean. *J. Geophys. Res.* 98, 2371–2383.
- Waite, T.D., Szymczak, R., Espey, Q.I., Furnas, M.J., 1995. Diel variations in iron speciation in northern Australian shelf waters. *Mar. Chem.* 50, 79–91.
- Wells, M.L., 1999. Manipulating iron availability in nearshore waters. *Limnol. Oceanogr.* 44, 1002–1008.
- Wells, M.L., Mayer, L.M., Guillard, R.R.L., 1991a. A Chemical method for estimating the availability of iron to phytoplankton in seawater. *Mar. Chem.* 33, 23–40.
- Wells, M.L., Mayer, L.M., Donard, O.F.X., de Souza Sierra, M.M., Ackelson, S.G., 1991b. The photolysis of colloidal iron in the oceans. *Nature* 353, 248–250.
- Wells, M.L., Price, N.M., Bruland, K.W., 1995. Iron chemistry and its relationship to phytoplankton: a workshop report. *Mar. Chem.* 48, 157–182.
- Wiebinga, C.J., de Baar, H.J.W., 1998. Determination of the distribution of dissolved organic carbon in the Indian sector of the Southern Ocean. *Mar. Chem.* 61, 185–201.
- Wilhelm, S.W., Trick, C.G., 1994. Iron-limited growth of cyanobacteria: multiple siderophore production is a common response. *Limnol. Oceanogr.* 39, 1979–1984.
- Wilhelm, S.W., Maxwell, D.P., Trick, C.G., 1996. Growth, iron requirements and siderophore production in iron-limited *Synechococcus* PCC 7002. *Limnol. Oceanogr.* 41, 89–97.
- Wu, J., Luther, G.W. III, 1995. Complexation of Fe(III) by natural organic ligands in the Northwest Atlantic Ocean by a competitive ligand equilibration method and a kinetic approach. *Mar. Chem.* 50, 159–177.
- Zhou, X., Wangersky, P.J., 1989. Production of copper-complexing organic ligands by the marine diatom *Phaeodactylum tricornutum* in a cage culture turbidostat. *Mar. Chem.* 26, 239–259.
- Zhou, X., Slauwenwhite, D.E., Pett, R.J., Wangersky, P.J., 1989. Production of copper-complexing organic ligands during a diatom bloom: tower tank and batch-culture experiments. *Mar. Chem.* 27, 19–30.
- Zhuang, G., Yi, Z., Wallace, G.T., 1995. Iron (II) in rainwater, snow, and surface seawater from a coastal environment. *Mar. Chem.* 50, 41–50.